

10/566358

USE OF ADCC-OPTIMIZED ANTIBODIES
FOR TREATING WEAK PATIENTS

This invention relates to the use of humanised or human chimeric monoclonal antibodies with a specific glycosylation having a strong interaction for the CD 16 receptor (FcgammaRIII) of the effector cells of the immune system as well as the property of inducing cytokine and interleukin secretion, for treating populations of patients responding poorly to existing treatments and having a particular polymorphism of their CD16.

Immunotherapy with monoclonal antibodies is becoming one of the most important and innovative strategies in medicine. However, the results obtained in clinical trials appear to be mixed. Indeed, in a number of treatments, the monoclonal antibody does not appear to be active and/or effective enough. Numerous clinical trials have been stopped due to a lack of efficacy and adverse effects incompatible with use as a clinical treatment. These two aspects are closely related, given that relatively inactive and/or ineffective antibodies are administered at high doses to compensate for the lack of

efficacy and obtain a therapeutic response. The administration of high doses then causes adverse effects. In addition, it is economically unviable.

These are major problems in the industry of
5 monoclonal antibodies, in particular humanised or human
chimeric antibodies. However, these problems are
exacerbated for particular populations of patients in
whom the therapeutic effect of the antibodies is
significantly lower by comparison to the average in the
10 population treated. These patients are commonly called
"low-responder patients".

It is known that cell cytotoxicity mediated by
antibodies requires binding of the Fab portion of the
antibodies to their target as well as an engagement of
15 their Fc domain with the Fc receptors of the effector
cells (RFc). On the NK cells, the receptor responsible
for this cytotoxicity is CD16.

On the basis this knowledge, studies have been
conducted in order to establish a correlation between
20 subpopulations of patients who are refractory (so-called
"low-responder" patients) to treatment with an antibody
specific to a condition to be treated, and CD16
polymorphism.

In a study of 2000 (Cartron G, Dacheux L, Salles G,
25 Solal-Celigny P, Bardos P, Colombat P, Watier H,
Therapeutic activity of humanized anti-CD20 monoclonal
antibody and polymorphism in IgG Fc receptor FcgammaRIIIa
gene, Blood. 2002 Feb 1; 99(3): 754-8), the composition
of the population of patients treated with Rituxan® was
30 analysed according to the CD16 polymorphism in position
158. It was composed of 20% FCGR3A-158V homozygotes
(patients homozygous for valine in position 158 of CD16),

35% FCGR3A-158F homozygotes (patients homozygous for phenylalanine in position 158 of CD16) and 45% FCGR3A-158V/F heterozygotes (patients heterozygous for valine/phenylalanine in position 158 of CD16). The ADCC activity as well as the clinical efficacy of the anti-CD20 Rituxan® antibodies are correlated with the CD16 polymorphism: the FCGR3A-158F homozygous or FCGR3A-158V/F heterozygous patients are less responsive to the Rituxan® treatment than FCGR3A-158V homozygous patients.

Thus, this study made it possible to demonstrate that the differences in response to treatments with therapeutic antibodies are associated with a CD16 polymorphism.

However, this study does not propose a solution for reducing the effect of this polymorphism in so-called "low-responder" patients treated with therapeutic antibodies. It does not provide a therapeutic treatment suitable for the treatment of low-responder patients.

In addition, other results have been obtained from clinical studies performed with anti-Rhesus antibodies, which have shown that the elimination of Rhesus positive erythrocytes by IgG3 anti-D was faster in FCGR3A-158F homozygous patients (Kumpel BM, De Haas M, Koene HR, Van De Winkel JG, Goodrick MJ. Clearance of red cells by monoclonal IgG3 anti-D in vivo is affected by the VF polymorphism of Fcγ3R (CD16). Clin Exp Immunol. 2003 Apr; 132(1): 81-6). However, the low number of patients studied does not enable a technical result to be drawn from this study.

The objective of the present invention is to provide antibodies effective for treating patients in whom

treatments with the antibodies currently available fail, or suffering from adverse effects.

The applicant has found that antibodies in which the Fc domain has a particular glycan structure with a cytotoxic activity particularly suitable for the treatment of so-called "low-responder" patients, which is not affected by the FCGR3A-158F homozygous or FCGR3A-158V/F polymorphism of amino acid 158 of CD16, unlike the antibodies produced in CHO of which the cytotoxic activity is affected by this polymorphism. In addition, the applicant has found that the antibodies according to the invention cause cytokine/chemokine production, which also contributes to the reinforcement of the therapeutic effect by stimulating effector mechanisms of the immune system in the patients treated.

The invention therefore proposes the use of antibodies according to the invention in the treatment of sub-populations of so-called "low-responder" patients. These antibodies have an ADCC activity and a cytokine and/or chemokine production up to 100 times greater than the antibodies available in current treatments.

Thus, the invention relates to the use of an optimised humanised or human chimeric monoclonal antibody, characterised in that:

- a) it is produced in a cell line selected for its properties of glycosylation of the Fc region of an antibody, or
- b) the glycan structure of the Fc region was modified *ex vivo*, and/or
- c) its primary sequence was modified so as to increase its interaction with the Fc receptors;

wherein said antibody has (i) an ADCC level via CD16 (FcγRIIIA) above 60%, 70%, 80% or preferably above 90% compared with the same antibody produced in a CHO line or a homologous antibody available on the market,
5 for the preparation of a drug intended for treating conditions in patients with the polymorphism of the homozygous FCGR3A-158V/F or FCGR3A-158F CD16 receptor and considered "low-responders" to treatments with the antibodies currently available.

10 For the purposes of the invention, the term "low-responder" patients refers to patients with a 20% to 50% reduction in the response to treatments with therapeutic antibodies by comparison with so-called "high-responder" patients, i.e. patients with a complete response
15 corresponding to the disappearance of all measurable clinical signs and symptoms of the disease.

For example, in the case of a study of the clearance of erythrocytes or of another cell type in the bloodstream, the term "low-responder" patients refers to
20 patients who have a significantly longer clearance by comparison with another group of patients. In the treatment of leukaemia, the following are differentiated:
* the high responders with a complete response corresponding to the disappearance of all measurable
25 signs and symptoms of the disease, with respect to the clinical exam and the biological laboratory data and radiographic exams. The reduction in the size of the largest tumours is greater than 75%.

* patients who respond partially are described in the
30 article Cheson BD et al, Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working

Group. J Clin Oncol. 1999 Apr; 17(4): 1244. Review.
Erratum in: J Clin Oncol 2000 Jun; 18(11): 2351.

* low-responders, corresponding to patients having a so-called stable condition, with at least 50% reduction of
5 the tumour mass, less than 25% increase in lesions and no new lesions. This group of patients also includes patients in whom no response is observed (progression of disease progressing to death).

Thus, for a subpopulation of so-called "low-
10 responder" patients, in relation to the polymorphism of amino acid 158 of CD16 (homozygous FCGR3A-158F or FCGR3A-158V/F) or another polymorphism associated with this CD16 polymorphism, the efficacy of the treatment is better with the optimised antibodies of the invention, and comes
15 close to that of so-called "high-responder" patients.

Indeed, the interaction of the receptor for the Fc of antibodies is different according to the CD16 polymorphisms (aa158), as the homozygous FCGR3A-158V phenotype has a better affinity than the homozygous
20 FCGR3A-158F and heterozygous FCGR3A-158V/F form. The antibodies according to the invention have a strong interaction with CD16, which may explain the fact that their functional activity is not or is only very slightly affected by the homozygous FCGR3A-158F or FCGR3A-158V/F
25 CD16 polymorphism.

The invention therefore addresses a particular population of patients with a CD16 polymorphism (homozygous FCGR3A-158F or FCGR3A-158V/F), in particular
patients in whom the treatment with the antibodies
30 currently available has failed and/or patients suffering from adverse effects justifying the administration of the optimised antibody of the invention.

The conditions treated with the antibody according to the invention are not limited to particular conditions, but include all conditions capable of being treated with monoclonal antibodies.

5 In addition to the very significant improvement in the CD16-type ADCC activity (FcγRIIIA), the antibody of the invention can be characterised in that it induces the secretion of at least one cytokine by a type of effector cell of the immune system expressing the CD16 receptor
10 more than 50 %, 100 % or preferably more than 200 % with respect to the same antibody produced in a CHO line or compared with a homologous antibody available on the market. The type of cytokine is selected from IL-1, IL-4, IL-12, IL-18, IL-21, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8,
15 IL-9, IL-10, IFN α , IFN β , TNF α , TGF β , IP10 and TNF, IFN γ .

 In the context of the invention, we show that antibodies having a strong interaction with CD16 have the advantage of inducing the production of cytokines, or chemokines, in particular the production of IFN γ . The two
20 aforementioned characteristics complement one another. Indeed, the production of IFN γ or other cytokines or chemokines, by effector cells, induced by the antibodies according to the invention, can reinforce the therapeutic effect by stimulating effector mechanisms of the immune
25 system in the patients treated. The mechanism of action of such a stimulation corresponds in particular to a positive autocrine regulation of the effector cells. The antibodies binding to CD16 induce a cytotoxic activity as well as the production of IFN γ or other
30 cytokines/chemokines, which, in the end, results in a further increase in the cytotoxic activity.

Preferably, this antibody has an ADCC level above at least 100% compared to the same antibody produced in a CHO line or a homologous antibody available on the market, and a level of production, of at least one cytokine by a
5 type of effector cell of the immune system expressing the CD16 receptor, greater than at least 100% with respect to the same antibody produced in a CHO line or a homologous antibody available on the market.

The cytokines of which the release is caused by
10 optimised antibodies are selected from interleukins, cytokines, chemokines, interferons and tumour necrosis factors (TNF).

Thus, the antibody according to the invention is capable of inducing the secretion of at least one type of
15 cytokine selected from IL-1, IL-4, IL-12, IL-18, IL-21, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IFN α , IFN β , TNF α , TGF β , IP10 and TNF, IFN γ , by the effector cells of the immune system.

Preferably, the antibody selected has the capacity
20 to induce the secretion of IFN γ or other cytokines/chemokines by the effector cells of the immune system expressing the CD16 receptor or more specifically IL2 by the CD16 Jurkat cell. The level of IFN γ or other cytokines and/or chemokines secreted expresses the
25 capacity for interaction of the Fc region of the antibody with CD16 and also expresses its capacity for binding to the antigen. The secretion of IFN γ or other cytokines and/or chemokines by the cells of the immune system can increase and/or induce the cytotoxic activity of the
30 effector cells (NK, monocytes, macrophage, polynuclear neutrophils, etc).

The effector cells can express an endogenous CD16 possibly modulable by cytokines and/or chemokines or growth factors or be transformed. The term transformed cell refers to a cell genetically modified so as to
5 express a receptor, in particular the CD16 receptor.

In a particular embodiment, the antibody of the invention is capable of inducing the secretion of at least one cytokine by a leukocyte, in particular the family of NK (Natural Killer) cells, or by cells of the
10 myelomonocyte line (monocytes-macrophages and dendrite cell).

Preferably, for the selection of antibodies, a Jurkat line transfected with an expression vector encoding the CD16 receptor as the effector cell is used.
15 This line is particularly advantageous because it is immortalised and is maintained in culture indefinitely. The level of IL2 secreted expresses the capacity for interaction of the Fc region of the antibody with CD16 and also expresses its capacity for binding to the
20 antigen.

Various Jurkat lines transfected with an expression vector encoding the CD16 receptor can be used as an effector cell, which lines each express a particular CD16.

In addition, the optimised antibody can be prepared
25 after having been purified and/or modified *ex vivo* with regard to the glycan structure of its Fc region. To this end, any chemical, chromatographic or enzymatic means appropriate for modifying the glycan structure of the antibody can be used. For example, it is possible to
30 purify antibodies obtained from various sources, to add one or more glycosyl transferase(s), in particular a galactosyl-transferase, to a reaction mixture, and to

incubate for a predetermined time until antibodies with the glycan structure described above are obtained.

The selection can be made of antibodies produced by cells commonly used to produce therapeutic antibodies, such as rat myeloma lines, in particular YB2/0 and its derivatives, human lymphoblastoid cells, insect cells, murine myeloma cells, hybridomas as well as eukaryotic cells such as, for example, yeast.

It is also possible to produce the antibodies in a genetically modified mammal cell line, for example genetically modified CHO, by introducing one or more sequence(s) expressing one or more glycosyl transferase(s), in particular selected from an enzyme involved in the modification of oligosaccharide chains in position 1 of the fucose alpha bonded to position 6 of the N-acetylglucosamine residue at the reductive end, in particular a 1,6-fucosyltransferase, and a galactosyltransferase. The selection can also be applied to the evaluation of antibodies produced by transgenic plants or transgenic mammals.

Therefore, the production in CHO serves as a reference (CHO being used in the production of drug antibodies) for comparing and selecting the production systems leading to the antibodies according to the invention. A comparison with polyclonal antibodies can also be useful in monoclonal antibody efficacy tests. Thus, for a subpopulation of so-called "low-responder" patients in relation to the polymorphism of amino acid 158 of CD16 or another polymorphism associated with this polymorphism, the efficacy of the treatment is better with the optimised antibodies of the invention, and is similar to that of so-called "high-responder" patients.

We show that the functional activity of the optimised monoclonal antibodies is related to that of therapeutic polyclonal antibodies. Thus, in some therapeutic trials, the polyclonal antibodies can be used as controls in tests on the efficacy of monoclonal antibodies of different origins. This makes it possible to select monoclonal antibodies intended for the treatment of subpopulations of low-responder patients.

Thus, in some therapeutic trials, the polyclonal antibodies can be used as controls in tests on the efficacy of monoclonal antibodies of different origins. This makes it possible to select monoclonal antibodies intended for the treatment of subpopulations of low-responder patients. Another alternative consists of performing a comparison with the antibodies available on the market, in particular antibodies being developed, antibodies for which marketing authorisation has been obtained, or antibodies for which the clinical trials were stopped, and shown to be ineffective or to produce adverse effects at the doses administered. Indeed, the modified antibodies of the invention are at least 100% more effective for activating the ADCC supported by effector cells of the immune system, which means lower administration doses than those used for the antibodies mentioned above, and, in this case, the possibility of treating patients in whom the antibodies currently available have proved to be ineffective.

In a preferred embodiment of the invention, the antibody can, in a first step, be selected for its capacity of interaction with the CD16 receptor, then tested and selected as described above for its properties of inducing the production of a cytokine, in particular

IL-2, by the Jurkat CD16 cells or IFN γ by the effector cells expressing CD16.

Such antibodies have this dual property of inducing ADCC via the CD16 and of inducing the production of IFN γ or other cytokines and/or chemokines by the cells of the immune system can increase and/or induce the cytotoxic activity of the effector cells (in particular, NK, monocytes, macrophage, and polynuclear neutrophils).

Thus, the invention relates to the use of the antibody defined above for the preparation of a drug intended for the treatment of conditions in patients homozygous for phenylalanine in position 158 of CD16 (FCGR3A-158F homozygotes) or patients heterozygous for valine/ phenylalanine in position 158 of CD16 (FCGR3A-158V/F).

Such antibodies according to the invention have a specific glycosylation. The antibodies according to the invention have a biantennary-type Fc domain, with short chains, low sialylation, non-intercalating terminal mannoses and GlcNAc of the attachment site and low fucosylation.

Thus, in another embodiment of the invention relates to the use of a humanised or human chimeric monoclonal antibody of which the glycan structure of the Fc domain of the antibody corresponds to a biantennary type, with short chains, low sialylation, non-intercalating terminal mannoses and GlcNAc of the attachment site, and low fucosylation for the preparation of a drug intended for the treatment of conditions in patients homozygous for phenylalanine in position 158 of CD16 (FCGR3A-158F homozygotes) or patients heterozygous for

valine/phenylalanine in position 158 of CD16 (FCGR3A-158V/F).

Indeed, this glycan form of the Fc domain of the antibody confers on the latter the properties of inducing
5 a strong DCC and the production of cytokines and/or chemokines as described above.

In this antibody, the level of intercalary GlcNac is non-zero. For example, compositions with a concentration over 60%, preferably over 80% for the forms G0 + G1 + G0F
10 + G1F, can be used given that the concentration in forms G0F + G1F is lower than 50%, preferably lower than 30 % for the preparation of a drug intended for the treatment of patients homozygous for phenylalanine in position 158 of CD16 (FCGR3A-158F homozygotes) or patients
15 heterozygous for valine/phenylalanine in position 158 of CD16 (FCGR3A-158V/F).

The antibodies mentioned above are preferably IgG1.

Another objective of the invention is to provide a therapeutic treatment method including the administration
20 of a humanised or human chimeric monoclonal antibody of which the glycan structure of the Fc domain of the antibody corresponds to a biantennary type, with short chains, low sialylation, non-intercalating terminal mannoses and GlcNAc of the attachment site and low
25 fucosylation, to patients homozygous for phenylalanine in position 158 of CD16 (FCGR3A-158F homozygotes) or patients heterozygous for valine/phenylalanine in position 158 of CD16 (FCGR3A-158V/F).

The patients are advantageously homozygous for
30 phenylalanine in position 158 of CD16 (FCGR3A-158F homozygotes).

Preferably, in the patients treated, the antibodies currently available resulted in therapeutic failure or the patients experienced adverse effects.

The dose of the antibody administered to the patient
5 is preferably 2 times, 5 times, and preferably 10 times, 25 times, 50 times or most preferably 100 times lower than a dose indicated with an antibody having the same specificity but different glycosylation or produced in a CHO line.

10 The dose of the antibody administered to the patient is advantageously between 2 and 5 times, between 5 and 10 times, between 5 and 25 times, between 5 and 50 times or preferably between 5 and 100 times lower than a dose of an antibody with the same specificity by different
15 glycosylation or produced in a CHO line.

In another embodiment of the invention, the antibody of the invention can be produced in rat myeloma cell lines, for example YB 2/0 (ATCC no. CRL 1662). Indeed, such cells enable a glycosylated antibody as
20 previously described to be obtained. Thus, in a complementary embodiment of the invention relates to the use of a humanised or human chimeric monoclonal antibody produced in a rat myeloma line, for example YB2/0 or one of its derivatives, for the preparation of a drug
25 intended for the treatment of patients having the homozygous form FCGR3A-158V/F or FCGR3A-158F of CD16, in particular patients in whom treatment with the antibodies currently available has failed, or suffering from adverse effects justifying the administration of the optimised
30 antibody of the invention. The invention preferably relates to the use of antibodies produced in rat myeloma cell lines, in particular YB2/0 and its derivatives. Said

antibodies having the dual property of inducing, via the homozygous form FCGR3A-158V/F or FCGR3A-158F of CD16, ADCC, as well as the production of IFN γ or other cytokines and/or chemokines by the cells of the immune system, which is capable of increasing and/or inducing the cytotoxic activity of effector cells (NK, monocytes, macrophage, polynuclear neutrophils, etc.) described as expressing the CD16 receptor, are used for the preparation of a drug intended for the treatment of a particular population of low-responder patients or patients in whom the treatment with the antibodies currently available has failed or suffering from adverse effects.

The antibody according to the invention, produced in rat myeloma lines, in particular YB2/0 or one of its derivatives, has the glycan structure of the fragment Fc as described above, the concentration in forms G0+G1+G0F+G1F and G0F+G1F as described above, and it induces a cytotoxicity by ADCC and the secretion of cytokines in the way described above.

The antibody of the invention can be directed against a non-ubiquitous antigen present in healthy donor cells (for example, the antibody is of anti-Rhesus human erythrocyte specificity), or an antigen of a pathological cell or an pathogenic organism for humans, in particular against an antigen of a cancer cell or a cell infected by a virus. It is advantageous to use the antibodies defined above for the treatment of cancers and infections by pathogenic agents for these patients.

The conditions requiring the administration of such antibodies in these patients include, for example, diseases selected from haemolytic disease of the newborn

and those escaping the immune response, in particular Sezary syndrome, chronic myeloid leukaemias, chronic lymphoid leukaemias (CLL-B), solid tumours, breast cancer, conditions related to the environment in particular
5 affecting people exposed to polychlorinated biphenyls, infectious diseases, in particular tuberculosis, chronic fatigue syndrome (CFS), parasitic infections such as, for example, schistosomes or paludism, in particular in pregnant women, and viral infections for targeting the
10 virus reservoir cells (HIV, HCV, HBV in particular) and the infected cells.

The conditions treated include conditions in which the antigen is poorly expressed (Rhesus D antigen on erythrocytes leukaemia, chronic lymphoid leukaemia B
15 (CLL-B), for example). "Poorly-expressed antigen" refers to a number of antigenic sites below 250,000, preferably below 100,000 or 50,000 and very advantageously below 10,000 or 5,000 per target cell.

In a particular embodiment of the invention
20 addresses patients with the homozygous FCGR3A-158V/F or FCGR3A-158V form of CD16 and having CLL-B. As the CD20 is very poorly expressed on these tumour cells, the use of anti-CD20 antibodies according to the invention for treating these patients is particularly advantageous.

25 The cancers concern most specifically cancers of HLA class-II positive cells, B-cell lymphomas, B-cell acute leukaemias, Burkitt's lymphoma, Hodgkin's lymphoma, myeloid leukaemias, chronic lymphoid leukaemias (CLL-B), T-cell lymphomas and leukaemias, non-Hodgkin's lymphomas
30 and chronic myeloid leukaemias.

The antibody according to the invention can be an anti-HLA-DR antibody or an anti-CD20.

In a particular embodiment of the invention, when the antibody is an anti-CD20, the antibody is advantageously administered at a dose below 187.5 mg/kg, at 75 mg/kg, at 37.5 mg/kg, 15 mg/kg, 7.5 mg/kg or preferably below 3.75 mg/kg. The dose administered is advantageously between 187.5 mg/kg and 75 mg/kg, or between 75 mg/kg and 37.5 mg/kg, between 75 mg/kg and 15 mg/kg, between 75 mg/kg and 7.5 mg/kg and preferably between 75 mg/kg and 3.75 mg/kg or between 15 mg/kg and 3.75 mg/kg.

This antibody advantageously has an ADCC level above 100% and an IL-2 production level by the CD16 Jurkat cell up to 1000% greater than that of Rituxan®.

The anti-CD20 of the invention can be produced in a rat myeloma line, in particular YB2/0.

In addition, by way of example, the antibodies according to the invention can be second generation antibodies corresponding to the antibodies currently available, listed in Table 1.

Table 1

Name and brand name of the antibody	Company	target	indication
Edrecoloma b PANOREX	Centocor	anti Ep-CAM	colorectal cancer
Rituximab RITUXAN	Idec Licensed to Genentech/ Hoffman la roche	anti CD20	B cell lymphoma thrombocytopeni a purpura
Trastuzuma b HERCEPTIN	Genentech Licensed to Hoffman la roche/ Immunogen	anti HER2	ovarian cancer
Palivizuma	Medimmune		RSV

b SYNAGIS	Licensed to Abbott		
Alemtuzuma b CAMPATH	BTG Licensed to Schering	anti CD52	leukaemia
ibritumoma b tiuxetan ZEVALIN	IDEC Licensed to Schering	anti CD20	NHL
Cetuximab IMC-C225	Merck/ BMS/ Imclone	anti HER1	cancers
Bevacizuma b AVASTIN	Genentech/ Hoffman la roche	anti VEGF	Lung, colorectal, kidney cancers
Epratuzuma b	Immumedics/ Amgen	anti CD22	cancers: non-Hodgkin's lymphoma
Hu M195Mab	Protein Design Labs	ND	cancers
MDX-210	Medarex/ Immuno- Designed Molecules	Bispecific HER2Neu/C D64	Breast, ovarian, prostate cancers
BEC2 Mitumomab	Imclone	anti GD3	Small cell lung carcinoma
Oregovomab OVAREX	Altarex	anti CA125	Ovarian cancer
Ecromexima b KW-2971	Kyowa-Hakko	anti GD	malignant melanoma
ABX-EGF	Abgenix	EGF	cancers
MDX010	Medarex	ND	cancers
XTL 002	XTL biopharmaceutical s	ND	anti-viral: HCV
H11 SCFV 4B5	viventia biotech	ND	cancers
XTL 001	XTL biopharmaceutical s	ND	anti-viral: HBV
MDX-070	MEDAREX	Anti-PSMA	Prostate cancer
TNX-901	TANOX	anti CD- 23	
IDEC-114	IDEC	Protein C inhibition	non-Hodgkin's lymphoma

Other antibodies can be selected from anti Ep-CAM, anti HER2, anti CD52, anti HER1, anti GD3, anti CA125, anti GD, anti GD2, anti CD-23 and anti Protein C; anti-KIR3DL2, anti-EGFR, anti-CD25, anti-CD38, anti-CD30, 5 anti-CD33, anti-CD44, inhibitor-specific anti-idiotypes, for example, coagulation factors, and anti-virals: HIV, HBV, HCV and RSV.

In a preferred embodiment the antibody is an anti-HLA-DR. This antibody has an ADCC level above 100% and a 10 level of production of IL2 by the CD16 Jurkat cell, or IFN γ by an effector cell of the immune system expressing the CD16 receptor of up to 1000 compared with the same antibody expressed in the CHO line, the expression line of Remitogen®.

15 The anti-HLA-DR of the invention can be produced in a rat myeloma line, in particular YB2/0.

The invention also relates to the use of an antibody described above for the production of a drug intended to induce the expression of IL-1, IL-4, IL-12, IL-18, IL-21, 20 IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IFN α , IFN β , TNF α , TGF β , IP10 and IFN γ by the natural effector cells of the immune system, which drug is useful in particular in the treatment of cancer and infections in patients in whom the antibodies currently available have 25 failed, or suffering from adverse effects justifying the administration of the optimised antibody of the invention, and in particular having the form V/F158 or F/F158 of CD16.

Other embodiments and advantages of the invention 30 will be described in the following examples, which should be considered to be illustrative and do not limit the scope of the invention.

Legends of the figures

Figure 1: ADCC activity of the anti-Rhesus D monoclonal antibody R297 (IgG1 T125 produced in YB2/0) and polyclonal antibodies WinRho in the presence of effector cells (PBMC: Peripheral Blood Mononuclear Cells) of different blood donors and polyvalent immunoglobulins (Tegeline 500 µg/ml).

Figure 2: ADCC induced by anti-Rhesus D antibodies (polyclonal antibodies WinRho, T125 produced in YB2/0 and T125 produced in CHO) on NK cells of donors of homozygous phenotype CD16 FCGR3A-158F.

Figure 3: ADCC induced by anti-Rhesus D antibodies (polyclonal antibodies WinRho, T125 produced in YB2/0 and T125 produced in CHO) on NK cells of donors of homozygous phenotype CD16 FCGR3A-158F.

Figure 4: Activation of homozygous FCGR3A-158F CD16 Jurkat by the anti-Rhesus D antibodies T125 expressed in YB2/0 and CHO.

Figure 5: Activation of homozygous FCGR3A-158V CD16 Jurkat by the anti-Rhesus D antibodies T125 expressed in YB2/0 and CHO.

Figure 6: Activation of homozygous FCGR3A-158F CD16 Jurkat induced by the anti-HLA-DR antibodies expressed in YB2/0 and CHO.

Figure 7: Activation of homozygous FCGR3A-158V CD16 Jurkat induced by the anti-HLA-DR antibodies expressed in YB2/0 and CHO.

30 Examples

Example 1: ADCC activity of the anti-Rhesus D monoclonal antibody R297 compared with the polyclonal anti-D antibodies on a group of 107 blood donors.

The respective capacities of the anti-Rhesus D R 297
5 monoclonal antibody and polyclonal anti-D antibodies to lyse erythrocytes in the presence of effector cells of different individual donors are compared (figure 1).

The effector cells come from a group of 107 blood donors. The mononuclear cells (PBMC) are isolated from a
10 blood bag by centrifugation on a Ficoll gradient (Pack Plus Pharmacia). The platelets are removed by centrifugation (190 g, 15 min) and the residual red blood cells are lysed with NH₄Cl. The cells are washed and resuspended at 8×10^7 cells/ml in IMDM. The red blood
15 cells obtained from therapeutic concentrates (group O, Rhesus +) are treated for 10 min with papain (1 mg/ml) then washed three times in a saline buffer and adjusted to a concentration of 4×10^7 /ml or 2×10^7 /ml (NK test) in IMDM.

20 The test is performed in a plate with 96 wells (NUNC). The culture supernatants or the purified antibodies (100 μ l to 200 ng/ml in IMDM + 0.5% FBS), the effector cells (25 μ l), the red blood cells (25 μ l) and the polyvalent immunoglobulins (Tegeline, LFB) (50 μ l)
25 are incubated for 16 h at 37 °C in a CO₂-enriched atmosphere. For the non-specific lysis, the effector cells are replaced with IMDM. After 16 h at 37 °C, the plates are centrifuged. 60 μ l of supernatants are collected and mixed with 60 μ l of 2.7 diaminofluorene
30 (DAF, Sigma).

After 5 min, the OD is measured at 620 nm.

The percentage of lysis is estimated by using a calibration curve obtained with different dilutions of red blood cells lysed with NH_4Cl , corresponding to 100%, 75%, 50%, 25% and 0% lysis, respectively.

5 On the basis of the genotypic study conducted on donors of the same geographic region, the present study estimates that the average distribution of the 107 donors with regard to the CD16 polymorphism is 27 FCGR3A-158F homozygotes, 20 FCGR3A-158V homozygotes and 60 FCGR3A-
10 158V/F.

The results show a wide variability in the capacity of the effector cells of the different subjects to induce lysis of the positive Rhesus erythrocytes, regardless of the antibodies tested. The antibodies expressed in the
15 YB2/0 cell line have a cytolytic activity comparable to that of the polyclonal antibodies, regardless of the donor studied, and consequently regardless of the CD16 polymorphism of the effector cells of the donor (see figure 1).

20

Example 2: ADCC efficacy of the antibodies produced in CHO and YB2/0 according to the CD16 polymorphism.

The same sequence encoding a specific IgG1 of the Rhesus D antigen was transfected into CHO and YB2/0 cell
25 lines. The antibodies were incubated with positive Rhesus erythrocytes (target cells) and NK cells from 6 different donors (3 FCGR3A-158V homozygotes and 3 FCGR3A-158F homozygotes) previously genotyped for their CD16 phenotype in position 158. The NK cells are isolated
30 using the magnetic bead separation technique (MACS) of Myltenyi. The NK cells are washed and resuspended at $2 \times 10^7/\text{ml}$ and/or $6 \times 10^7/\text{ml}$ in IMDM. The red blood cells are

adjusted to a concentration of 2×10^7 /ml in IMDM. The Tegeline is replaced by the IMDM. Aside from these modifications, the test is identical to the ADCC test with PBMC.

5 The cytotoxic activity of the antibodies on the erythrocytes (ADCC) was evaluated (figure 2 and figure 3).

 The antibody produced in the CHO line induced a lower lysis than the antibody produced in YB2/0, regardless of the donor's phenotype. The R297 antibody
10 expressed in the YB2/0 line induced, from the lowest concentrations, a stronger cytolytic activity. At the maximum concentration of 25 ng/ml, the two antibodies induced the same percentage of ADCC.

 At concentrations below 25 ng/ml, the difference in
15 lysis between the antibody produced in CHO and that produced in YB2/0 is greater in the homozygous FCGR3A-158F donors than in the homozygous FCGR3A-158V donors. At a concentration of 2.5 ng/ml, in the presence of homozygous FCGR3A-158V NK cells, the antibody produced by
20 CHO induced 54% lysis while that produced by YB2/0 induced 89% lysis, that is, a 56% increase. By contrast, at the same concentration, in the presence of homozygous FCGR3A-158F NK cells, the antibody produced by CHO induced only 22% lysis while that produced by YB2/0
25 induced 74% lysis, that is a 236% increase.

 The antibody expressed in the YB2/0 line therefore proved to be a better product for treating the patients giving a low lysis with the antibodies produced in CHO. Thus, the difference in ADCC activity between the FCGR3A-
30 158V and homozygous FCGR3A-158F patients is lower with the antibody expressed in YB2/0 (89% and 74%) by

comparison with that observed with the antibody expressed in CHO (56% and 22%).

The optimised antibodies have a response that therefore appears to be less dependent on the polymorphic
5 forms of the CD16.

In addition, the monoclonal antibody expressed in YB2/0 always induces a lysis greater than or equal to the polyclonal antibodies.

10 Example 3: Comparison of the activation of homozygous FCGR3A-158F CD16 Jurkat cells and homozygous FCGR3A-158F CD16 Jurkat cells induced by anti-Rhesus antibodies produced in CHO and YB2/0 respectively: evaluation of IL2 production.

15 This test estimates the capacity of the antibodies to bind to the CD16 receptor (Fc gamma RIII) expressed on the CD16 Jurkat cells and to induce the secretion of IL2.

The same sequence encoding an IgG1 (T125) specific to the Rhesus D antigen was transfected into the CHO and
20 YB2/0 cell lines. The antibodies are incubated with positive Rhesus erythrocytes (target cell) and CD16 Jurkat cells (effector cells). Two types of Jurkat cells were used: 1- cells transfected with the gene encoding an RFc bearing the amino acid phenylalanine F in position
25 158 (form F), 2- cells transfected with the gene encoding an RFc bearing the amino acid valine V in position 158 (form V). The amount of cytokine (IL2) secreted by the CD16 Jurkat cells was measured by ELISA.

In a 96-well plate in mixture:

30 Antibody: 50 µl of a dilution of 50; 37.5; 25; 18.75; 12.5; 9.4; 6.25; 3.125 ng/ml in IMDM (Iscove's Modified Dulbecco's) Medium 5% FBS (foetal bovine serum)

- PMA 50 μ l of a dilution at 40 ng/ml in IMDM 5% FBS
- Red blood cells treated with papain. 50 μ l at 8×10^6 /ml in IMDM 5% FBS
- Jurkat CD16. 50 μ l at 2×10^6 /ml in IMDM 5% FBS

5 Incubation 1 night at 37 °C

Then centrifugation of plates, collection of 100 μ l of supernatants and assay of IL2 with the commercial kit (Quantikine of R/D). Reading at 450 nm.

10 The antibody expressed in the YB2/0 line is capable of inducing a higher secretion of IL2, contrary to the antibody expressed in CHO, regardless of the CD16 phenotype (figures 4 and 5).

The antibody produced in CHO does not induce the secretion of IL2 from homozygous FCGR3A-158F CD16 Jurkat
15 and only a very small production of IL2 with the homozygous FCGR3A-158V form.

As one of the special features of the Rhesus system is the low expression of the antigen at the membrane surface, it appears that under these conditions, the
20 antibody expressed in the YB2/0 line is a much better product for activating the effector cells with the homozygous form FCGR3A-158F of CD16 which do not appear to be capable of being activated with the antibody produced in CHO. As regards the homozygous form FCGR3A-
25 158V, very small amounts of antibodies produced by YB2/0 (<1.56) make it possible to induce an activation comparable to that obtained with higher concentrations of antibodies produced in CHO (12.5 ng/ml).

With the homozygous form FCGR3A-158F and at a
30 concentration of 12.5 ng/ml, the antibody produced in CHO induces a secretion of IL2 (18 pg/ml) lower than 2% of that induced by the antibody produced in YB2/0 (1435

pg/ml). This corresponds to an increase of more than 7000 %, when the antibody produced in YB2/0 is used, by comparison with the antibody produced in CHO.

With the homozygous form FCGR3A-158V and at a concentration of 12.5 ng/ml, the antibody produced in CHO induces a secretion of IL2 (869 pg/ml) lower than 8% of that induced by the antibody produced in YB2/0 (12312 pg/ml). This corresponds to an increase of more than 1300%, when the antibody produced in YB2/0 is used, by comparison with the antibody produced in CHO.

Example 4: Comparison of the activation of homozygous FCGR3A-158F CD16 Jurkat cells and homozygous FCGR3A-158V Jurkat cells induced by two anti-HLA-DR antibodies expressed in CHO and YB2/0, respectively: evaluation of the secretion of IL2.

The same sequence encoding an IgG1 specific to the HLA-DR antigen was transfected into the CHO and YB2/0 cell lines. The antibodies are incubated with Raji cells (positive HLA-DR target cell) and CD16 Jurkat cells (effector cells). Two types of Jurkat cells were used: 1- cells transfected with the gene encoding an RFc bearing the amino acid phenylalanine F in position 158 (form F), 2- cells transfected with the gene encoding an RFc having the amino acid valine V in position 158 (form V). The quantity of cytokines (IL2) secreted by the CD16 Jurkat cells was measured by ELISA.

In 96-well plates in mixture:

Antibody: 50 µl of a dilution of 50; 37.5; 25; 18.75; 12.5; 9.4; 6.25; 3.125 ng/ml in IMDM 5% FBS

- PMA 50 µl of a dilution at 40 ng/ml in IMDM 5% FBS

- Raji cells: 50 µl at 6×10^5 /ml in IMDM 5% FBS

- Jurkat CD16. 50 μ l at 20×10^6 /ml in IMDM 5% FBS

Incubation 1 night at 37 °C

Then centrifugation of plates, collection of 100 μ l of supernatants and assay of IL2 with the commercial kit
5 (Quantikine of R/D). Reading at 450 nm.

The antibody expressed in the YB2/0 line is capable of inducing a higher secretion of IL2, contrary to the antibody expressed in CHO, regardless of the CD16 phenotype (figures 6 and 7).

10 Unlike the Rhesus system on erythrocytes, the expression of the HLA-DR antigen at the membrane surface of the Raji cell is not low (200,000 to 400,000 copies). Under these conditions, it appears that the antibody expressed in the YB2/0 line is a much better product for
15 activating the effector cells with the form F158 as well as V158 of CD16, and more specifically with low antibody concentrations. Thus, the concentration of 1.56 ng/ml, the antibody produced in CHO induces a secretion of IL2 (2410 pg/ml) lower than 15% of that induced by the
20 antibody produced in YB2/0 (16952 pg/ml). This corresponds to an increase of more than 600% when the antibody produced in YB2/0 by comparison with the antibody produced in CHO.

At a concentration of 12.5 ng/ml, the antibody
25 produced in CHO induces a secretion of IL2 (14597 pg/ml) lower than 45% of that induced by the antibody produced in YB2/0 (34823 pg/ml). This corresponds to an increase of more than 100% when the antibody produced in YB2/0 is used, by comparison with the antibody produced in CHO.

30 The antibody expressed in the YB2/0 line is therefore a much better product for inducing the secretion of cytokines of effector cells with the

polymorphic form V158 (FCGR3A-158V homozygotes) and F158 (FCGR3A-158F homozygotes).